KOPLIK (H.)

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THE RAPID BACTERIOLOGICAL AND CLINICAL DIAGNOSIS OF DIPHTHERIA.

By HENRY KOPLIK, M. D.

From a clinical standpoint the rapid and satisfactory diagnosis of diphtheria, fortified by bacteriological culture test, is of the utmost importance. It is highly desirable in every case to satisfy our minds, as early as possible, whether the disease we are dealing with is that in which the Klebs-Loeffler bacillus is present or in which we have simply a streptococcus diphtheria or, if possible, a mixed infection. Clinically it has been amply demonstrated that the types of angina in which the Klebs-Loeffler bacillus is found do not include simply the membranous formation. We have all gradations of angina, from the throat in which membrane is absolutely absent, to that in which the fully formed membrane exists (Escherich, Koplik, Feer, Park, Flexner, Welch). With our present methods of therapy and the demands of prophylactic hygiene, it has become more and more imperative to shorten the time within which a positive bacteriological diagnosis of the nature of a given case of angina or membranous formation in the throat may be

made (true diphtheria of Loeffler, or diphtheroid angina). It is also desirable to have a simple method, a rapid and easily attainable means of diagnosis, within the reach of the general practitioner, not only in out-of-the-way communities but even in large cities. In the latter places, in spite of the presence of well-equipped health stations, economic and hygienic reasons are tending to compel every physician to acquaint himself with the technics of the early diagnosis of diphtheria.

I have made a series of studies with the ordinary serum tubes with a view to fixing the shortest space of time within which a diagnosis of true Loeffler diphtheria may be made beyond a reasonable doubt. I am now able to fix this time by tube culture at two and a half to three hours from the time the tube-culture medium is placed in the incubator. As is well known, if we take a piece of membrane or secretion from the tonsils or fauces of a suspected case of diphtheria and spread this in the usual way between two cover glasses, stain with Loeffler's blue solution and examine, we see, if the case is one of Loeffler diphtheria, a few isolated bacilli here and there having the characteristic form and stain of the Loeffler bacillus. In a vast number of cases the diagnosis, even if membrane is present, remains a matter of speculation on account of the paucity in the numbers of bacilli and the absence of the characteristic grouping of these bacilli, to which we will refer at length. In cases of diphtheria of the larynx, or even of the fauces, in which there is absolutely no visible membrane, a diagnosis by cover glass is impossible. It is in just such cases that an early and rapid diagnosis is most desirable. Moreover, I am convinced that the methods now most in vogue, by which the culture tubes are allowed to remain

in the incubator fully eighteen hours or twelve hours, give the ubiquitous streptococci and staphylococci sufficient time, at an elevated temperature and such a favorable medium as blood serum, to outgrow the bacillus diphtheriæ in numbers, and thus obscure the final diagnosis if, indeed, it does not make it impossible. It is true, as Loeffler first showed, that the serum remains the best medium for the cultivation of the bacillus diphtheriæ, for on this medium alone can the bacillus hold its own against the growth of other bacteria. Yet there are many cases in which we only succeed in obtaining a relatively small number of the Loeffler bacilli on our swab, as compared with the streptococci, and in the serum tube. It is just in these cases, as will be seen, that the early diagnosis shows the bacilli to be present, whereas after twelve to eighteen hours the streptococci or staphylococci will have outgrown them and obscured the diagnosis. This must account for the number of cases in which no result is attained by the old methods in which pronounced membrane is present in the fauces.

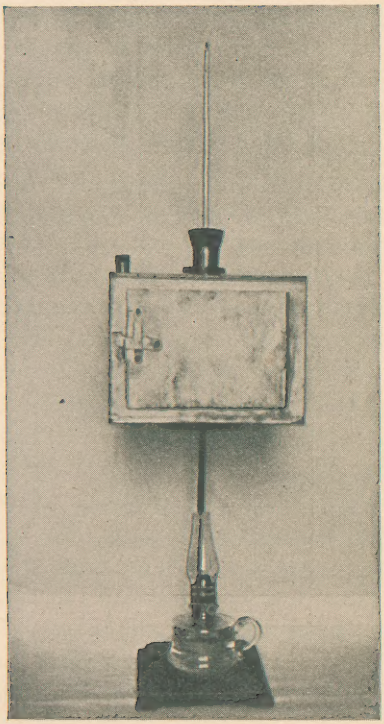
As has been intimated, the medium which I have employed in my studies to cultivate the bacillus diphtheriæ for clinical diagnosis is blood serum, for after all has been said it is the most convenient and certain medium, and may not be easily replaced in the future by anything more favorable to the growth of the bacillus diphtheriæ. During the first hours of the sojourn of the serum-culture tube in the incubator it can be seen that the bacillus diphtheriæ not only thrives but does this at a rate exceeding that of the other bacteria. But the streptococci present in any given case must vary largely. Therefore, in the early stages of the culture, even if the streptococcus in material numbers surpasses that of the

bacilli, the bacilli increasing in numbers still can be easily diagnosticated. Later on, if the bacilli are few in number, the streptococcus growth spreads so rapidly over the surface of the serum as to obscure or cover the bacilli in certain cases.

The sterilized swab on the end of a stout wire, as now in general use in New York, is the most practical means of taking the specimen of secretion or membrane from the throat.

THE INCUBATOR.—As an incubator, a small water oven is used, the interior chamber of which measures $4 \times 5 \times 6$ inches—just large enough to accommodate small or large test tubes. The whole is mounted, as shown in the accompanying cut, on an ordinary retort stand. It is supplied with a thermometer which is fixed in the central opening and shows the temperature of the interior. It may be supplied with a small thermometer at the lateral opening showing the temperature of the water, but this is hardly needed. It will be seen that the oven is of the most primitive construction, and can be obtained in all supply stores; it is not armed with an external felt or asbestos coating to prevent radiation, as the short period of its use scarcely calls for this precaution. The simplicity will speak for its use in the physician's office laboratory, especially as it can be taken apart and put up at a moment's notice. To prepare the oven for use, it is filled with water, or kept so prepared; the temperature of the internal chamber is raised rapidly to 37°–38° Celsius by means of a Bunsen flame —this takes about five minutes; as the temperature reaches 37° the flame is taken away, for the temperature continues to mount to 38° C., and at this point a small oil lamp, as shown in the cut, re-

places the Bunsen flame. The lamp, turned quite low, will maintain the internal temperature quite constant at 38° for any length of time. For three hours' heating



we need scarcely disturb the flame, as it takes care of itself. For longer periods it may need occasional watching.

METHOD OF PROCEDURE.—The patient is placed facing the light. A tongue depressor having brought the

fauces into view, the sterilized swab is rubbed gently over the suspected membrane or patch in the throat, or even the inflamed tonsils where no membrane exists. The swab is then carefully and evenly rubbed on the slanted surface of the blood-serum culture medium in the test tube.

The inoculated tube is then placed on the floor of the oven in a horizontal position. This is done in order that the glass tube and culture medium may become rapidly warmed to the temperature of the internal chamber of the incubator. If this culture tube is taken out at the end of two hours and a half, we can see by means of a magnifying glass that the surface of the serum has already become coated with a distinct growth of minute bacterial colonies, both in the Loeffler bacillus cases and those cases in which streptococci or staphylococci are present. At this early stage all the colonies, whether streptococcus or bacilli colonies, have the same transparency, and can not be differentiated as they can after twenty-four hours' or eighteen hours' growth, when the colonies of the bacilli of Loeffler have a whiter appearance as compared with those of the streptococci.

At the end of four hours the growth on the surface of the serum is perceptible even to the naked eye. It can be perceived if the serum is held between the light and the eye as a thin film on the surface of the serum, if the latter is transparent enough. At the end of five hours the growth is a very palpable one. From a clinical standpoint the culture gives diagnostic and very satisfactory results when examined at the end of two and a half to three hours. These results are not improved upon, as will be shown by the results obtained by a longer sojourn in the incubator. The bacteria have only increased in

numbers. In some cases it will be shown that to wait puts us at a distinct disadvantage.

The surface of the serum, which has been taken out of the incubator at the end of two hours and a half or three hours at the latest, is now carefully scraped with a sterilized platinum wire bent at its extreme point into a minute crook. The scraping is made in a longitudinal direction and should include, if possible, all the surface where we think the growth has occurred. No attempt is made to pick out any colonies. The scrapings are then spread on a cover glass with a drop of water, in the usual manner. The specimen is dried in the flame and stained with the Loeffler alkaline blue.

THE OPTIMITY OF GROWTH OF THE KLEBS-LOEFFLER BACILLUS.—It has been shown by Roux, in his very early work on diphtheria, that the bacillus diphtheriæ attains its greatest luxuriance of growth in artificial media below the temperature of 39.5° C. At this temperature, however, and above it, the bacillus shows a diminished vigor, and in a few days it ceases to grow. At 40° the retarding effect of the elevated temperature is more distinctly apparent.

In my studies I found that the best results are attained if the temperature of the internal chamber is kept ranging from 37° C. to 38° C. Above this temperature the serum does not show a satisfactory growth of bacilli, and these bacilli show the swollen and clubbed forms more distinctly. The involution forms are more common at the temperature of 40° or above. The growth, however, of bacilli is less vigorous than that of the streptococci or staphylococci at 40° or 41° or 42° C. The method, therefore, of procedure is to force the Loeffler bacilli to increase rapidly in numbers at a tem-

perature corresponding to their optimity of growth, 38° C.; not to overstep this point, and yet not work below 37° C.

Classes of Cases in which this Method has been fully Tested.

Class A: Case I.—Laryngeal symptoms, no visible membrane, laryngeal cough and breathing. Male infant, a year and a half old, sick for three days with croupy cough and breathing. Retraction at each inspiration of the episternal notch and peripneumonic groove; consolidation of the apex of the left lung (pneumonic); no membrane. Culture in incubator an hour, no diagnosis; culture in incubator two hours, streptococci; culture in incubator four hours, groups of Loeffler bacilli in abundance. Tube in incubator eighteen hours: the streptococci had overgrown the bacilli so that the latter were not apparent, as in the four-hour specimen. Thus, between the second and fourth hour bacilli were produced in sufficient numbers to enable a diagnosis.

Class B, Lacunar Diphtheria of the Tonsils: Case II.—Boy, aged five years and a half, sick with fever; croupy cough for three days; tonsils enlarged; the lacunar plugs evident; no membrane. Some of the plugs quite large. Culture in incubator three hours: almost pure culture of Loeffler bacilli; culture in incubator eighteen hours (overnight): bacilli only more abundant.

Class C, Membranous Cases: Case III.—Boy, aged three years; yellow-white membrane on the left tonsil; no other history. Culture in incubator an hour, no positive diagnosis; culture in incubator two hours, abundant Loeffler bacilli; culture in incubator four hours, abundant Loeffler bacilli; culture in incubator eighteen hours, streptococci more abundant, yet bacilli abundant also.

Case IV.—Boy, five years and a half old, has been ill three days with symptoms of a severe sore throat.

Right tonsil is covered with a grayish coating. Culture in incubator two hours and a half, pure culture of Loeffler bacilli; culture at end of eighteen hours, same bacilli, only more abundant.

CASE V.—Boy, three years old, has been ill only one day with fever and a sore throat. Both tonsils are covered with a greenish membrane. Tube in incubator at 41°–42°, after three hours we find Klebs-Loeffler bacilli in abundance. There are numbers of involution forms—swollen, club-shaped bacilli of uneven stain. Culture after eighteen hours showed that the bacilli had been outgrown by the streptococci; no bacilli found in specimens examined.

CASE VI.—Female infant, a year and eight months old, ill one day. The left tonsil coated with a greenish membrane, but slight adenitis. Culture in incubator two hours and a half, almost a pure culture of Klebs-Loeffler bacilli, few chains of streptococci; culture after eighteen hours (overnight), diphtheria bacilli predominant, few streptococci.

CASE VII.—Male child, two years and a half old, ill one day. Both tonsils covered with a greenish membrane, slight adenitis. Culture in incubator two hours and a half, Klebs-Loeffler bacilli in numerous groups, few streptococci; culture after eighteen hours, Klebs-Loeffler bacilli still more abundant, streptococci few.

CLASS D, CASES OF ANGINA WITH THE EXANTHEMATA: CASE VIII, MEASLES.—Female child, three years and a half old, just recovering from measles, pigmented mottling of skin still to be made out. For the past three days has had a croupy voice and cough. Tonsils enlarged but no membrane to be seen. Culture in incubator two hours, streptococci; culture in incubator four hours, streptococci; culture in incubator eighteen hours, streptococci.

CASE IX, SCARLET FEVER.—Female child, four years and a half old, has been ill for the past three days with slight diarrhœa and vomiting. There is a febrile movement; a slight efflorescence on the upper part of the chest; a marked tonsillitis, with a membranous forma-

tion, on the left tonsil. Diagnosis: scarlet fever and diphtheria. Culture tube in incubator two hours and a half, streptococci; culture tube in incubator four hours, streptococci; culture tube in incubator eighteen hours, streptococci.

CLASS E, CASES WHICH ARE NOT CHARACTERISTIC: CASE X.—Female, seven years old, has been ill with symptoms of angina one day. There is a yellow plug quite large in the left tonsil. Culture in the incubator two hours and a half, staphylococci; culture in the incubator eighteen hours, staphylococci; no bacilli in early or late culture.

CASE XI.—Female child, two years and three months old, sick for three days with a nasal discharge and sore throat; tonsils quite large; uvula coated with a hazy, bluish pellicle at the base; no distinct membrane; test taken from tonsils and uvula. Culture in incubator two hours and a half, streptococci; culture in incubator four hours, streptococci; culture in incubator eighteen hours, streptococci.

CASE XII.—Male infant, thirteen months old, has enlarged tonsils and streaks of yellow muco-pus on the tonsils; no distinct membrane. Culture in incubator three hours, streptococci; culture in incubator four hours, streptococci; culture in incubator eighteen hours, streptococci.

CASE XIII.—Female, aged five years, has for some days past had a discharge from the nose. There is a bluish-white discoloration inside the right nostril, like a thin pellicle. Tonsils show lacunar plugs, left tonsil a grayish coating of mucus. Cultures from nose and throat in incubator two hours and a half, streptococci; culture in incubator eighteen hours, streptococci; no bacilli found.

CASE XIV.—Female child, three years old, has been ill with cough and sore throat three days. The left tonsil shows a yellow spot; no distinct membrane. Culture in incubator three hours, streptococci; culture in incubator four hours, streptococci; culture in incubator eighteen hours, streptococci.

CLASS F, SURGICAL WOUND DIPHTHERIA: CASE XV. —Female child, three years old, suffering from a large granulating surface on the left buttock, the result of a severe burn. This surface had become coated with a thick, yellow, tenacious material, in some places dry and of a greenish hue; in places it looked like a pseudo-membrane. Culture tube in incubator two hours and a half showed a very luxuriant growth of bacilli not in any way resembling the Klebs-Loeffler bacilli.

DIAGNOSIS.—In order to fix a diagnosis, we have required in all cases, in addition to general character of size, etc.—

(a) An abundant bacterial growth, not isolated bacilli.

(b) The characteristic grouping of the Klebs-Loeffler bacillus in groups of pairs or fours, side by side, or more so arranged.

(c) Peculiarities of unevenness in staining with Loeffler alkaline solution of methyl blue.

(d) The presence of involution forms, club shapes, etc.

If the reader will refer to Cases I and V he will see that the contention of the writer in the first part of this paper, concerning the desirability of making a diagnosis early in the culture growth, is well founded, especially in cases (as Case I) where no characteristic membrane was present in the fauces. Here it must have occurred that but few bacilli were introduced by the swab into the serum tube as compared with the relatively enormous streptococcus material on the swab. The tubes examined early showed quite plainly, and without any reasonable fault, distinct groups of Klebs-Loeffler bacilli, fully convincing for a clinical diagnosis. These same tubes, examined after a prolonged incubation, showed

streptococci or other bacteria. The bacilli, if present, could only be found by prolonged search. The streptococcus material was clearly so abundant that though starting on an equal basis, or rather at a disadvantage, it had finally at an advanced temperature and additional time overgrown the bacilli.

Our method, which will apply to the difficult as well as the apparent cases of diphtheria, is to force the increase in number of the Klebs-Loeffler bacilli at the highest temperature favorable to them, 37° to 38° C., and to examine the culture tube two hours and a half to three hours after placing in the incubator. The streptococci and staphyloccci and sarcinæ have then as yet not attained considerable growth, and the Klebs-Loeffler bacilli, if present, can easily be found.

66 East Fifty-eighth Street.